Caspase-3 is necessary and sufficient for cleavage of protein synthesis eukaryotic initiation factor 4G during apoptosis

Martin Bushell[a], Linda McKendrick[a], Reiner U. Jänicke[b], Michael J. Clemens[c,*], Simon J. Morley[a]

[a] *Biochemistry Group, School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK*
[b] *Institute of Molecular and Cell Biology, The National University of Singapore, 30 Medical Dr., Singapore 117609, Singapore*
[c] *Department of Biochemistry, Cellular and Molecular Sciences Group, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK*

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Abstract Induction of apoptosis BJAB cells is accompanied by the rapid cleavage of protein synthesis eukaryotic initiation factor 4G and the appearance of a fragment of approximately 76 kDa. Inhibition of apoptotic proteases (caspases) has previously been shown to prevent the cleavage of eukaryotic initiation factor 4G. In MCF-7 breast carcinoma cells, which are deficient in caspase-3, eukaryotic initiation factor 4G is not cleaved but in vivo expression of caspase-3 restores eukaryotic initiation factor 4G cleavage following induction of apoptosis. Recombinant caspase-3 can also cleave eukaryotic initiation factor 4G to yield the 76 kDa fragment both in cell extracts and when the eukaryotic initiation factor 4G is presented in a purified eukaryotic initiation factor 4F complex. These results indicate that caspase-3 activity is necessary and sufficient for eukaryotic initiation factor 4G degradation.

Key words: Apoptosis; Caspase; Eukaryotic initiation factor 4G

1. Introduction

The mammalian polypeptide chain eukaryotic initiation factor 4G (eIF4G) plays a crucial role in the mechanism by which the translation of all cellular mRNAs takes place by acting as a bridge between other components of the ribosomal initiation complex (reviewed in [1,2]). Two forms of eIF4G have been identified, termed eIF4GI and eIF4GII [3]. eIF4GI is a single polypeptide chain of 154 kDa [4] which exists at least partly in the form of a trimeric complex with the mRNA cap binding protein eIF4E and the ATP-dependent RNA helicase eIF4A, constituting the initiation factor eIF4F [5]. As well as having domains for the binding of eIF4E and eIF4A, the factor possesses binding sites for eIF3 [6] and the poly(A) binding protein (PABP) [7–11]. Interaction of PABP with eIF4GI may allow functional association of the 3′ end of an mRNA with the 5′ end [9,10]. Since it is likely that eIF4GII has similar properties, the reason for the existence of two different eIF4G species is not presently understood.

The association of eIF4G with eIF4E markedly enhances the binding of the latter to the mRNA cap [12]. This interaction is inhibited by the eIF4E binding proteins (4E-BPs) which compete with eIF4G for binding to eIF4E [13–16]. Release of eIF4G from eIF4E in the presence of the binding protein 4E-BP1 reduces the susceptibility of eIF4G to cleavage by the foot and mouth disease virus L protease [17], suggesting that eIF4E may cause a conformational change in eIF4G that exposes the L protease cleavage site.

Apoptosis (programmed cell death) involves a series of cellular events resulting in characteristic morphological changes, alterations in membrane permeabilities, the selective cleavage of protein targets, the endonucleolytic fragmentation of DNA and, ultimately, loss of cell viability [18–21]. A large body of research has characterised the properties of the apoptotic proteases (caspases) involved in these processes, together with the nature of several caspase substrates that are cleaved during the execution of the apoptotic programme [21–27]. We have recently reported that eIF4G is very susceptible to degradation during apoptosis induced by serum deprivation, anti-Fas (CD95) stimulation, etoposide or cycloheximide treatment in BJAB and Jurkat lymphoma cell lines and that proteolysis of the factor to produce distinct cleavage fragments under these conditions is mediated by caspase activity [28,29]. Moreover, protein synthesis is strongly inhibited under conditions of apoptosis and, at least in the case of anti-Fas treatment, this inhibition can be prevented by incubation with the cell permeable caspase inhibitor, z-VAD.FMK [29]. Here, we show that caspase-3 is both necessary and sufficient for cleavage of eIF4G and that the caspase-dependent degradation of eIF4G is not influenced by the association of the latter with eIF4E.

2. Materials and methods

2.1. Materials

Tissue culture materials were purchased from Life Technologies, cycloheximide was obtained from Sigma-Aldrich, the caspase inhibitor z-VAD.FMK was from Alexis Biochemicals and purified caspase-3 was purchased from Autogen Bioclear (UK). Bacterial extracts containing recombinant forms of caspase-1 and caspase-3 were a gift from Dr. S. Goodbourn (St George's Hospital Medical School, London, UK).

2.2. Cell culture and induction of apoptosis

The EBV-negative Burkitt's lymphoma cell line BJAB and the breast cancer cell lines MCF-7 (caspase-3 deficient) and MCF-7.3.28 (stably transfected to express caspase-3) were cultured as described in

*Corresponding author. Fax: (44) (181) 725 2992.
E-mail: m.clemens@sghms.ac.uk

Abbreviations: eIF, eukaryotic initiation factor; m^7GTP, 7-methylguanosine triphosphate; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; MOPS, (3-(*N*-morpholino))propanesulfonic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether); 4E-BP, eIF4E binding protein

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[28] and [30,31], respectively. Apoptosis was induced with cycloheximide (100 μg/ml) [28], tumour necrosis factor (30 ng/ml) plus cycloheximide (10 μg/ml) or staurosporine (1 μM) [30,31]. The times of treatment are given in the individual figure legends.

2.3. Preparation and analysis of cell extracts

Cytoplasmic extracts of cells were prepared for immunoblotting as described previously [28]. Nuclei were removed by centrifugation for 5 min in a microcentrifuge at 4°C. Samples were subjected to electrophoresis on sodium dodecyl sulfate (SDS) polyacrylamide gels and the proteins transferred to polyvinylidene difluoride membranes (Millipore) using a semi-dry blotting apparatus (Hoefer). Equal amounts of total protein were loaded on the gels. Antibodies to a region of eIF4G encompassing amino acids 920–1396 and to α-fodrin were as described previously [28–31]. Immunoblots were either developed using alkaline phosphatase-linked secondary antibodies with nitroblue tetrazolium as substrate or were analysed by enhanced chemiluminescence.

2.4. Preparation of recombinant proteins

The human eIF4G cDNA sequence from plasmid pSKHFC1 [32] was subcloned as an *Eco*RI fragment into pFastBac (Life Technologies). The *Xenopus* eIF4E cDNA sequence from plasmid pKS-41b (a kind gift from Dr. N. Sonenberg, McGill University, Montreal, Canada) was modified using the oligonucleotide sequence 5′-TCA CAT CCA TCT ACC ATG GCG GCC GTG GAA-3′ to include a *Nco*I site at the initiator AUG and then subcloned into pFastBacHtA (Life Technologies) as an *Nco*I-*Eco*RI fragment. Recombinant baculoviruses were then constructed from these shuttle vectors as per the manufacturer's instructions (Life Technologies). Initiation factor eIF4F was purified from *Sf*9 insect cells co-infected for 72 h with recombinant baculoviruses expressing eIF4G and eIF4E. The eIF4A component of this eIF4F originated from the insect cells. Cells were harvested and resuspended in 5 ml per 100 ml culture of lysis buffer (50 mM (3-(*N*-morpholino))propanesulfonic acid (MOPS), pH 7.2, 100 mM NaCl, 5 mM EDTA, 5 mM ethylene glycol-bis(β-aminoethyl ether) (EGTA), 5 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM benzamidine, 10 μM E-64 and 10 μg/ml leupeptin), lysed by the addition of IGEPAL (Sigma) to 1% (by volume) and clarified by centrifugation at 15 000×*g* for 20 min at 4°C. The supernatant was applied to 7-methyl-guanosine triphosphate (m^7)GTP-Sepharose resin, washed in buffer (20 mM MOPS, pH 7.2, 50 mM NaCl, 5 mM EDTA, 5 mM EGTA, 5 mM DTT, 1 mM PMSF, 1 mM benzamidine, 10 μM E-64 and 10 μg/ml leupeptin) and uncomplexed eIF4E and the eIF4F complex were eluted with 0.1 mM m^7GTP in the same buffer. eIF4F was separated from uncomplexed eIF4E by chromatography using MonoQ FPLC resin (Pharmacia) using a linear gradient of 50–500 mM NaCl (in 20 mM MOPS, pH 7.2, 100 μM EDTA, 2 mM EGTA, 0.05% (by volume) Tween-20, 7 mM 2-mercaptoethanol), as described previously [33]. Recombinant 4E-BP1 and foot and mouth disease virus L protease were prepared as described previously [17,33,34].

3. Results

3.1. Caspase-3 activity is required for cleavage of eIF4G in vivo

We have previously reported that the caspase inhibitors z-VAD.FMK and z-DEVD.FMK block the cleavage of eIF4G in both BJAB and Jurkat cells treated with several different inducers of apoptosis [28,29]. These data suggested that caspase activity was required for eIF4G cleavage, but did not establish whether the initiation factor was a direct substrate for any particular caspase. We have taken advantage of the existence of the MCF-7 cell line that is deficient in caspase-3 [30,31] to investigate the requirement for this enzyme in vivo during the cellular response to two different treatments that induce apoptosis. Fig. 1 shows that in MCF-7 cells, neither treatment with the combination of tumour necrosis factor (TNF) plus cycloheximide (lane 1 versus lanes 2 and 3) nor exposure to the protein kinase inhibitor staurosporine (lane 1 versus lanes 4 and 5) caused any loss of full-length eIF4G or

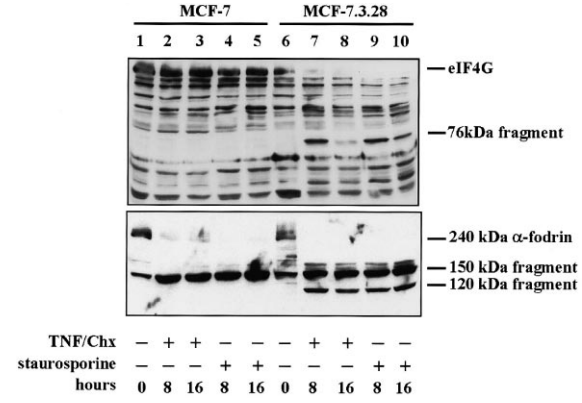

Fig. 1. Caspase-3-dependence of eIF4G cleavage in MCF-7 breast cancer cells. MCF-7 (caspase-3 deficient) and MCF-7.3.28 cells (stably transfected to express caspase-3) were incubated with or without TNF-α plus cycloheximide or staurosporine for the times indicated. Total cytoplasmic extracts were prepared and analysed by immunoblotting for eIF4G and α-fodrin. The bands corresponding to full-length eIF4G, its 76 kDa cleavage product and α-fodrin and its 150 and 120 kDa cleavage products are indicated. The data are from a single experiment but are representative of those obtained on three separate occasions.

the appearance of the 76 kDa fragment. In contrast, both conditions enhanced the degradation of the 240 kDa structural protein α-fodrin to give a 150 kDa product, which is mediated by the activation of calpain and not caspases [30]. However, when MCF-7 cells that were stably transfected to express caspase-3 (MCF-7.3.28) were subjected to the same treatments, eIF4G was cleaved to give the 76 kDa product (Fig. 1, lane 6 versus lanes 7 and 8 and lanes 9 and 10) and α-fodrin, a known substrate for caspase-3, was cleaved to yield a characteristic 120 kDa fragment [30]. Together, these data indicate that caspase-3 activity is essential for the cleavage of eIF4G in response to inducers of apoptosis. The 76 kDa fragment appears to be relatively unstable in these cells since its level declined substantially between 8 and 16 h after induction of apoptosis (Fig. 1, lane 7 versus lane 8 and lane 9 vsrsus lane 10).

Although caspase-3 is essential for eIF4G breakdown during apoptosis, the data presented in Fig. 1 do not tell us whether there is direct cleavage of the initiation factor by this enzyme. To investigate this, initially, we incubated cytoplasmic extracts of BJAB cells in vitro with bacterial cell extracts expressing either recombinant caspase-3 or recombinant caspase-1. Under these conditions, caspase-3 caused the cleavage of eIF4G to yield the characteristic 76 kDa fragment (Fig. 2A, lane 1 versus lane 3 and Fig. 2B, lane 3 versus lane 5). In contrast, incubation with caspase-1 (Fig. 2A, lane 1 versus lane 2) or a control bacterial extract (data not shown) failed to induce cleavage of eIF4G and did not affect the ability of caspase-3 to cleave the factor (Fig. 2A, lane 3 versus lane 4). We have also examined the effect of these bacterially expressed caspases on the cleavage of recombinant, purified eIF4G, co-expressed in baculovirus-infected insect cells with eIF4E. These data indicate that the bacterial cell extract expressing recombinant caspase-3 will cleave eIF4G presented in this form to yield the 76 kDa fragment (Fig. 2A, lane 5 versus lane 7 and Fig. 2B, lane 7 versus lane 8), but again, no specific cleavage was observed with caspase-1 (Fig. 2A, lane 5 versus lane 6). We have also used highly purified caspase-3 in these

assays. The data in Fig. 2B (lane 3 versus lane 6 and lane 7 versus lane 9) indicate that purified caspase-3 will cleave the eIF4G that is in cytoplasmic extracts or is presented as a purified protein. A direct comparison of the products of caspase-3-mediated eIF4G cleavage in vitro with those that appear in apoptotic cells shows precise electrophoretic co-migration of the 76 kDa protein (Fig. 2B, lanes 4–6). However, as shown in Fig. 2A and B, additional products of eIF4G cleavage with apparent sizes of 150 and 120 kDa were evident when purified eIF4F was used as the substrate for caspase-3. These novel fragments of eIF4G constitute only minor immunoreactive bands when apoptotic cell extracts or control cell extracts incubated with caspase-3 are analysed, suggesting that the larger fragments are unstable in vivo. Using antiserum to a different region of eIF4G, similar incomplete cleavage with caspase-3 in vitro was seen when a ribosomal salt wash fraction from HeLa cells was used as a source of eIF4G [35]. To address this in more detail, we examined the time course of appearance of the degradation products of eIF4G when purified eIF4F was incubated with caspase-3 in vitro (Fig. 2C). Under these assay conditions, eIF4G was cleaved within 15 min to yield the 150, 120 and 76 kDa products and there was an obvious precursor-product relationship between the full-length protein and these fragments.

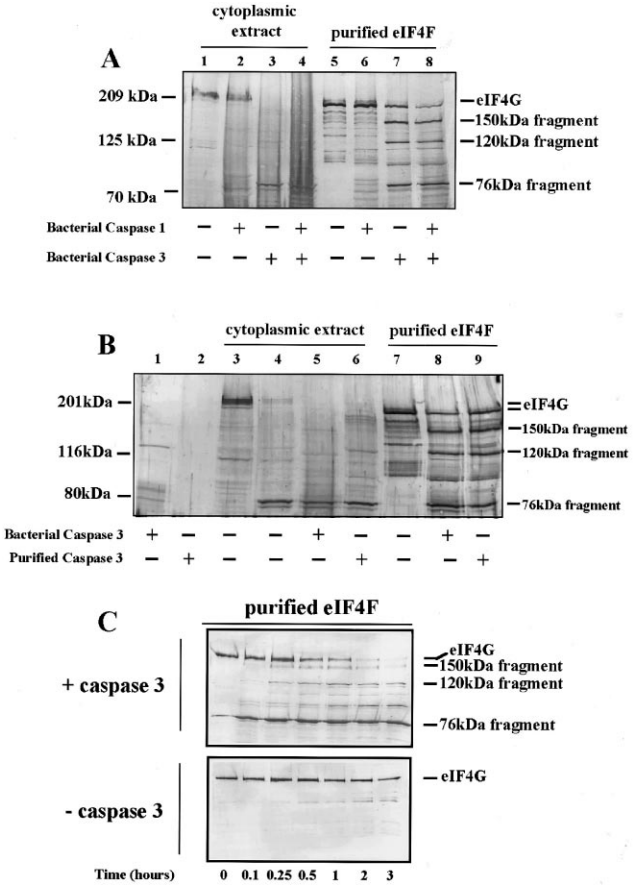

Fig. 2. Cleavage of eIF4G by recombinant caspase-3 in vitro and comparison of the products with those observed in apoptotic cells. A: A cytoplasmic extract was prepared from control BJAB cells and initiation factor eIF4F was purified from insect cells infected with recombinant baculoviruses expressing human eIF4G and eIF4E, as described in Section 2. Each preparation was incubated for 1 h at 30°C with or without extracts from bacteria expressing caspase-1 or caspase-3. The samples were then analysed for the integrity of eIF4G by SDS gel electrophoresis and immunoblotting. Molecular mass markers are indicated on the left and the bands corresponding to full-length eIF4G and its specific cleavage products are indicated on the right. B: Cytoplasmic extracts were prepared from control and apoptotic (8 h cycloheximide-treated) BJAB cells and eIF4F was purified from baculovirus-infected insect cells as above. Samples were incubated with or without extracts from bacteria expressing caspase-3 or with purified recombinant caspase-3 (20 μg/ml), as indicated, for 1 h at 30°C and then analysed as in A. Lanes 1 and 2: caspase-3 preparations alone; lanes 3, 5 and 6: control cell extract; lane 4: apoptotic cell extract; lanes 7–9: purified eIF4F. Molecular mass markers are indicated on the left and the bands corresponding to full-length eIF4G and its specific cleavage products are indicated on the right. C: Purified eIF4F (containing approximately 1 μg/ml of eIF4G) was incubated in the absence (lower panel) or presence (upper panel) of purified caspase-3 (5 μg/ml) for the times indicated. The samples were then analysed for the integrity of eIF4G by SDS gel electrophoresis and immunoblotting. Bands corresponding to full-length eIF4G and its specific cleavage products are indicated on the right. These data are from a single experiment but are representative of those obtained on three separate occasions.

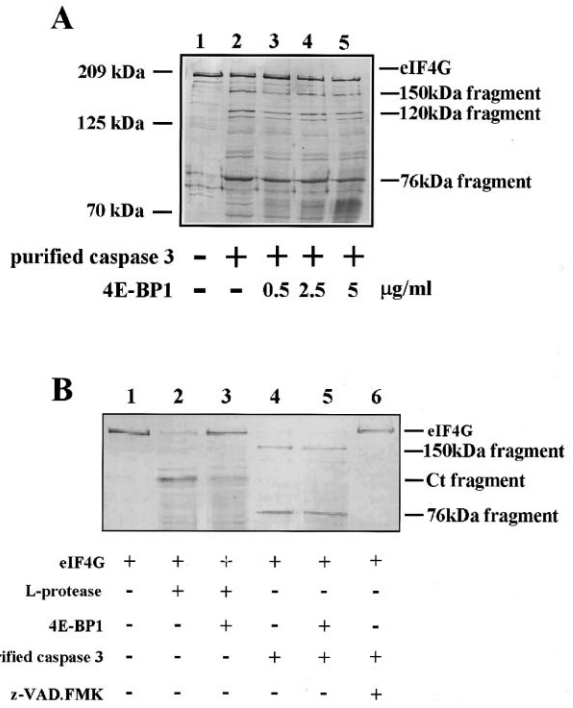

Fig. 3. Cleavage of eIF4G by caspase-3 is not inhibited by 4E-BP1. A: Initiation factor eIF4F (containing approximately 1 μg/ml eIF4G) was purified from baculovirus-infected insect cells as in Fig. 2 and pre-incubated alone or with recombinant 4E-BP1 (0.5, 2.5 and 5 μg/ml) for 10 min and then incubated for 30 min with or without caspase-3 (5 μg/ml) at 30°C, as indicated. The samples were then analysed for the integrity of eIF4G by SDS gel electrophoresis and immunoblotting. Molecular mass markers and the bands corresponding to full-length eIF4G and its specific in vitro cleavage products are indicated. B: Purified eIF4F (containing approximately 0.4 μg/ml eIF4G) was pre-incubated in the absence or presence of 4E-BP1 (25 μg/ml) or the caspase inhibitor z-VAD.FMK (100 μM) for 10 min at 30°C as indicated and was then further incubated for 1 h at 30°C with or without caspase-3 (20 μg/ml) or foot and mouth disease virus L protease (0.03 μg/ml). The samples were analysed as in A. Bands corresponding to full-length eIF4G, the C-terminal cleavage product generated by L protease (Ct) and the caspase-specific cleavage products are indicated. These data are from a single experiment but are representative of those obtained on three separate occasions.

3.2. The eIF4E binding protein 4E-BP1 does not prevent cleavage of eIF4G by caspase-3 in vitro

Previously, we have shown that the proteolytic cleavage of eIF4G by the foot and mouth disease virus L protease was prevented by the eIF4E binding protein 4E-BP1 in the reticulocyte lysate system [17]. These data suggested that the L protease cleavage site was inaccessible until a change in conformation of eIF4G was induced by the binding of eIF4E. We have used the in vitro cleavage system described in Fig. 2 to investigate whether the ability of 4E-BP1 to compete with eIF4G for binding to eIF4E alters the susceptibility of eIF4G to degradation by caspase-3. Fig. 3 shows that incubation of recombinant eIF4G with purified caspase-3 in vitro yielded the characteristic 150, 120 and 76 kDa cleavage fragments (Fig. 3A, lane 1 versus lane 2 and Fig. 3B, lane 1 versus lane 4). Addition of 4E-BP1 at concentrations of up to 5 μg/ml neither enhanced nor inhibited the ability of caspase-3 to cleave eIF4G in vitro (Fig. 3A, lane 2 versus lanes 3–5 and Fig. 3B, lane 4 versus lane 5). The caspase-3-dependence was demonstrated by the finding that z-VAD.FMK completely prevented the cleavage of eIF4G (Fig. 3B, lane 5 vrsus lane 6). To demonstrate that 4E-BP1 was active in these assays, we monitored the effect of L protease on the cleavage of eIF4G. Under the same assay conditions, 4E-BP1 was able to inhibit the appearance of the distinct Ct fragment generated by L protease cleavage [17] (Fig. 3B, lane 2 versus lane 3).

4. Discussion

Recently, we and others [28,29,35] have shown that protein synthesis initiation factor eIF4G is a target for caspase-mediated cleavage during apoptosis. Similar results were obtained when the integrity of the second form of eIF4G, eIF4GII [3], was analysed with specific antisera (M. Bushell and S. Morley, data not shown). In this paper, we have presented in vivo data indicating that eIF4G cleavage during apoptosis requires the participation of caspase-3 (Fig. 1). MCF-7 cells lacking this enzyme due to a functional deletion of the CASP-3 gene were unable to degrade eIF4G in response to treatment with TNF-α plus cycloheximide or staurosporine, although they can undergo some other aspects of apoptosis under these conditions [31]. Transfection and stable expression of caspase-3 restored the eIF4G cleavage activity. Although MCF-7 cells also lack some other caspase activities [30,31], caspase-3 alone is sufficient in vitro to produce the same 76 kDa eIF4G cleavage fragment as is observed in apoptotic BJAB, MCF-7.3.28 or Jurkat cells [28,29]. The enzyme also resulted in the appearance of larger fragments with apparent sizes of approximately 150 and 120 kDa. These fragments of eIF4G, which also appear in vivo [28,35], are less prominent than the 76 kDa product and are probably transient intermediates in the formation of the 76 kDa protein (Fig. 2 and data not shown). The conclusion that caspase-3 is both necessary and sufficient to cause site-specific proteolysis of eIF4G is consistent with our previous evidence that the caspase-3 inhibitor, z-DEVD.FMK, is able to prevent eIF4G cleavage in vivo [28,29]. Another recent study has also reported that caspase-3 is able to cleave eIF4G in vitro in preparations of initiation factors from HeLa cells [35].

In view of the inhibitory effect of recombinant 4E-BP1 towards cleavage of eIF4G mediated by the Foot-and-Mouth-Disease Virus L protease ([17] and Fig. 3B), we tested whether the same would be true for caspase-3-induced cleavage. A preparation of 4E-BP1 which was capable of protecting eIF4G from L protease-mediated degradation had no effect on the characteristic pattern of eIF4G cleavage caused by caspase-3. This suggests that the association of eIF4E with eIF4G is not required for the latter to be a substrate for caspase-3, although it does enhance the ability of eIF4G to be cleaved by L protease. The two enzymes utilise distinct cleavage sites in the eIF4G sequence (M. Bushell et al., manuscript submitted) and produce proteolytic fragments that are clearly distinguishable (Fig. 3B). It therefore seems likely that only the L protease site and not the caspase-3 site(s) is rendered more exposed by the binding of eIF4E to eIF4G.

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References

[1] Merrick, W.C. and Hershey, J.W.B. (1996) in: Translational Control (Hershey, J.W.B., Mathews, M.B. and Sonenberg, N., Eds.), pp. 31–70, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
[2] Morley, S.J., Curtis, P.S. and Pain, V.M. (1997) RNA 3, 1085–1104.
[3] Gradi, A., Imataka, H., Svitkin, Y.V., Rom, E., Raught, B., Morino, S. and Sonenberg, N. (1998) Mol. Cell. Biol. 18, 334–342.
[4] Yan, R., Rychlik, W., Etchison, D. and Rhoads, R.E. (1992) J. Biol. Chem. 267, 23226–23231.
[5] Merrick, W.C. (1994) Biochimie 76, 822–830.
[6] Lamphear, B.J., Kirchweger, R., Skern, T. and Rhoads, R.E. (1995) J. Biol. Chem. 270, 21975–21983.
[7] Tarun Jr., S.Z. and Sachs, A.B. (1996) EMBO J. 15, 7168–7177.
[8] Le, H., Tanguay, R.L., Balasta, M.L., Wei, C.C., Browning, K.S., Metz, A.M., Goss, D.J. and Gallie, D.R. (1997) J. Biol. Chem. 272, 16247–16255.
[9] Tarun Jr., S.Z., Wells, S.E., Deardorff, J.A. and Sachs, A.B. (1997) Proc. Natl. Acad. Sci. USA 94, 9046–9051.
[10] Piron, M., Vende, P., Cohen, J. and Poncet, D. (1998) EMBO J. 17, 5811–5819.
[11] Imataka, H., Gradi, A. and Sonenberg, N. (1998) EMBO J. 17, 7480–7489.
[12] Haghighat, A. and Sonenberg, N. (1997) J. Biol. Chem. 272, 21677–21680.
[13] Mader, S., Lee, H., Pause, A. and Sonenberg, N. (1995) Mol. Cell. Biol. 15, 4990–4997.
[14] Haghighat, A., Mader, S., Pause, A. and Sonenberg, N. (1995) EMBO J. 14, 5701–5709.
[15] Altmann, M., Schmitz, N., Berset, C. and Trachsel, H. (1997) EMBO J. 16, 1114–1121.
[16] Matsuo, H., Li, H.J., McGuire, A.M., Fletcher, C.M., Gingras, A.-C., Sonenberg, N. and Wagner, G. (1997) Nat. Struct. Biol. 4, 717–724.
[17] Ohlmann, T., Pain, V.M., Wood, W., Rau, M. and Morley, S.J. (1997) EMBO J. 16, 844–855.
[18] Hale, A.J., Smith, C.A., Sutherland, L.C., Stoneman, V.E.A., Longthorne, V.L., Culhane, A.C. and Williams, G.T. (1996) Eur. J. Biochem. 236, 1–26.
[19] Rowan, S. and Fisher, D.E. (1997) Leukemia 11, 457–465.
[20] Nagata, S. (1997) Cell 88, 355–365.
[21] Salvesen, G.S. and Dixit, V.M. (1997) Cell 91, 443–446.
[22] Fearnhead, H.O., Dinsdale, D. and Cohen, G.M. (1995) FEBS Lett. 375, 283–288.
[23] Tewari, M., Quan, L.T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D.R., Poirier, G.G., Salvesen, G.S. and Dixit, V.M. (1995) Cell 81, 801–809.

[24] Enari, M., Talanian, R.V., Wong, W.W. and Nagata, S. (1996) Nature 380, 723–726.
[25] Cohen, G.M. (1997) Biochem. J. 326, 1–16.
[26] Longthorne, V.L. and Williams, G.T. (1997) EMBO J. 16, 3805–3812.
[27] Ohta, T., Kinoshita, T., Naito, M., Nozaki, T., Masutani, M., Tsuruo, T. and Miyajima, A. (1997) J. Biol. Chem. 272, 23111–23116.
[28] Clemens, M.J., Bushell, M. and Morley, S.J. (1998) Oncogene 17, 2921–2931.
[29] Morley, S.J., McKendrick, L. and Bushell, M. (1998) FEBS Lett. 438, 41–48.
[30] Jänicke, R.U., Ng, P., Sprengart, M.L. and Porter, A.G. (1998) J. Biol. Chem. 273, 15540–15545.
[31] Jänicke, R.U., Sprengart, M.L., Wati, M.R. and Porter, A.G. (1998) J. Biol. Chem. 273, 9357–9360.
[32] Joshi, B., Yan, R.Y. and Rhoads, R.E. (1994) J. Biol. Chem. 269, 2058.
[33] Rau, M., Ohlmann, T., Morley, S.J. and Pain, V.M. (1996) J. Biol. Chem. 271, 8983–8990.
[34] Ohlmann, T., Rau, M., Pain, V.M. and Morley, S.J. (1996) EMBO J. 15, 1371–1382.
[35] Marissen, W.E. and Lloyd, R.E. (1998) Mol. Cell. Biol. 18, 7565–7574.